Second Quarterly Progress Report

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Neurophysiology Studies of Stimulated Auditory Prostheses

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Introduction

Neural prosthetic devices are artificial extensions to the body that restore or supplement nervous system function that was lost during disease or injury. Particular success has been realized in cochlear prostheses development. The devices bypass damaged hair cells in the auditory system by direct electrical stimulation of the auditory nerve. Stimulating discrete spiral ganglion cell populations in cochlear implant users' ears is similar to the encoding of small acoustic frequency bands in a normal-hearing person's ear. In contemporary cochlear implants, however, the injected electric current is spread widely along the scala tympani and across turns. Consequently, stimulation of spatially discrete spiral ganglion cell populations is difficult. One goal of implant device development is to design cochlear implants that stimulate smaller populations of spiral ganglion cells. In contrast to electrical stimulation, extreme spatially selective stimulation is possible using light.[1-5] Therefore, the goal is to develop and build optical cochlear implant prostheses to stimulate small populations of spiral ganglion cells. Steps towards this objective include (1) quantify the optical parameters that allow for safe spiral ganglion cell stimulation over extended periods of time, (2) characterize the fundamental spatial and temporal properties of optical stimulation of the auditory nerve, (3) determine the spatial resolution for laser stimulation. By accomplishing the first three goals within the first three years, we will be able (4) to build and implant the first animal cochlear implant electrode for long-term safety studies during years four and five. Also, the results will provide a basic set of parameters that can be used for other neural interfaces that use optical radiation to stimulate neurons.

During the last quarter we continued working on Step I: Quantify the parameters that allow the safe use of laser radiation for auditory nerve stimulation. The objectives for Step I are: Acute experiments are made in normal hearing and in long-term deafened gerbils. An optical fiber is placedafter surgical access to the cochlea close to the modiolus. The optical fiber is coupled to an optical radiation source. While the auditory system is stimulated with light pulses, compound action potentials are recorded at the round window for different stimulus parameters: radiation wavelength, pulse length, pulse repetition rate, increasing optical energy, extended stimulation times, different diameters of the optical fiber between 50 and 600 μm, variable fiber distances and orientations from the spiral ganglion cells in the modiolus, and for different locations of optical fiber placement along the cochlea. Measuring the peak-to-peak amplitude of the optically evoked potential serves to monitor cochlear function. The CAP amplitude decreases when cochlear damage occurs. The results provide the safe stimulation parameters for optical stimulation of the auditory system.

Summary of activities from January 31, 2007 to April, 2007:

Publications resulting form the activities (September 2006-April 2007) copies of the new manuscripts are enclosed and posters are accessible on the internet. Publications during the last quarter are in blue)

Peer reviewed

1. Izzo, A.D., M. Bendett, M.E. Jansen, E., Jim Webb, Heather Ralph, Walsh, Jr., J.T., Richter, C.-P. (2007) Optical Parameter Variability in Laser Nerve Stimulation: a study of pulse duration, repetition rate, and wavelength. IEEE Transactions on Biomedical Engineering (in press).
2. Teudt, I.U., Nevel, A., Izzo, A.D., Walsh, Jr., J.T., Richter, C.-P. (2007) Optical stimulation of the facial nerve – a new monitoring technique? The Laryngoscope (in press).

Proceeding paper

1. Izzo A.D., Littlefield, P., Walsh, Jr., J.T., Webb, J., Ralph, H., Bendett, M., Jansend, D.E. and Richter, C.-P. (2007) Laser stimulation of auditory neurons at high repetition rate, SPIE Vol. 6435, 64350R-1 - 64350R-7.

Abstracts

1. Suh, E., Agnella D. Izzo, A.D., Walsh Jr., J.T., Richter, C.-P. (2007) The role of Transient Receptor Potential channels in neural activation. Abstr. Assoc. Res. Otolaryngol. 30, 109.
2. Izzo, A.D. Lin, A., Oberoi, M., Walsh, Jr.[1], J.T., and Richter, C.-P. (2007) Tone-on-light masking reveals spatial selectivity of optical stimulation in the gerbil cochlea. Abstr. Assoc. Res. Otolaryngol. 30, 446.
3. Littlefield , L., Izzo, A.D, Mundi, J., Walsh, Jr., J.T., Jansen, D.E., Bendett, M., Webb, J. Ralp, H., Richter, C.-P. (2007) Laser stimulation of the auditory nerve stimulation is possible at high repetition rates. Abstr. Assoc. Res. Otolaryngol. 30, 356.
4. Bayon, R., Otting, M., Izzo, A.D., Walsh Jr., J.T., Richter, C.-P. (2007) Optical stimulation of the auditory nerve before and after deafening in adult gerbils. CLO, April 9, Chicago

Personnel involved

Paid by the grant

Izzo, A.: post-doctoral student
Otting, M.: Technician
Richter, C.-P.: PI

Part of their training

Littlefield, P.: Neurootology Fellow
Lin, A.: Medical student
Suh, E.: Pre-medical student
Nevel, A.: Pre-medical student
Bayron, R.: Resident at the Department of Otolaryngology
Bradley, A.: Student at the Department of Biomedical Engineering

Technical activities

We received the multi-channel recording system from Plexon, setup the system, and tested the system with artificial data. Successful experiments with guinea pigs have not been accomplished yet, but will be made during the next (third) quarter.

Midwinter Meeting of the Association of Research in Otolaryngology (Denver). During the meeting the results of our experiments were presented in three posters. Important feedback has been gained through the discussions. Furthermore, an in depth discussion about the laser stimulation with Dr. Harris, D. at the University of Washington resulted in the following goals:

1.) we offered to provide Dr. Harris data of the optical stimulation that would allow him to do Monte Carlo simulations to estimate the heat distribution in the tissue.
2.) we offered Dr. Harris to consult in using the laser to stimulate neurons.
3.) we expressed that we are open for collaborations and to share our data.

Initiated by Aculight, through Dr. Bendett, we offered Dr. Chertoff to help using the laser to stimulate and then subsequently damage selected tissue. We suggested to Dr. Cherhoff to come to Chicago and participate in experiments so he can see how we use the laser to stimulate the auditory system.

March, 26, 2007 we met with our colleagues from Vanderbilt and with Aculight for a workshop on stimulation of neural tissue using optical radiation (see program below).

First Optical Nerve Stimulation Research Meeting
Monday March 26, 2007

Vanderbilt University – Conference Room of the Free Electron Laser Center

Attending

Jay Walsh, Duco Jansen, Anita Mahadevan-Jansen, Gaja Mahadevan, Mark Bendett, Claus-Peter Richter, Agnella Izzo, Johnathan Cayce, Johnathan, Ashok Choudhury, Jonathon Wells, Mike Remple, Chris Kao, Peter Konrad

Invitation

We aim to have an open meeting with ample time for discussions, brainstorming. We do not want/expect polished conference-like presentations. We should spend significant time on failures (i.e. experiments that were tried but did not work as expected) as we could probably learn much from those. Specific meeting goals:

1) get everyone up to speed on who is doing what such that we can collective bundle our efforts rather than duplicate each others work.
2) evaluate where we stand in terms of unraveling the underlying mechanisms of optical stimulation of neural tissue.
3) challenges and obstacles translating stimulation concept to stimulation in the Central Nervous System.
4) identify optimal parameters for specific applications.

Schedule:

9 am	Welcome and Introductions – Duco Jansen
9.15-9.45 am	Stimulation of peripheral motor nerves – Jonathon Wells
9.45-10.00	Discussion
10.00-10.30	Coffee Break
10.30-11.00	Stimulation of sensory nerves – Agnella Izzo
11.00-11.30	Discussion
11.30-11.45	Stimulation in spinal cord – Gajendiran Mahadevan
11.45-12.00	Discussion
12.00-12.15	Update on clinical studies – Jonathan Wells
12.15-12.30	Discussion
12.30-1.30	Lunch – Lobby Vanderbilt MFEL Center
1.30-2.00	Laser Developments – Mark Bendett
2.00-2.30	Discussion
2.30-2.45	Stimulation in Brain slices – Jonathan Cayce
2.45-3.00	Stimulation in mouse cerebellum – Duco Jansen

3.00-3.15	Discussion
3.15-3.45	Break
3.45-4.15	Stimulation in the cochlea – Claus-Peter Richter
4.15-4.30	Discussion
4.30-5.30	Mechanisms (anyone who has any data on this, please present slides to kick off the discussion)
6.30 p.m.	Dinner at Sunset Grill

End of Meeting

We maintained our Website at:
http://www.oto-hns.northwestern.edu/Richter%20Lab/index.htm.

Scientific activities

Summary

1. Single fiber experiments were continued and existing data were analyzed. At present, the data are not complete and experiments will continue during the next quarter.
2. During the last quarter animals had been deafened to validate the laser parameter in longterm deafened animals. The experiments are still in progress and have not been completed.
3. With a novel laser from Aculight we were able to stimulate neurons with 1μs-radiation pulses. With this laser we also test a different radiation wavelength.
4. Selectivity of optical stimulation has been determined by tonal masking.

Single fiber measurements in gerbils

Activities of single auditory nerve fibers were recorded, while the cochlea was stimulated with optical radiation (see last quarter's progress report). Most of the data have been analyzed and have been presented at the ARO meeting. Discussions at the poster reinforced our current attempts to record form a single fiber before and after deafening of the animal. The experiments are extremely difficult. Although no single neuron could be maintained during the entire deafening process, it was possible to record from single auditory nerve fibers after deafening the animals by using the laser to stimulate the neurons. After deafening the cochlea with neomycin, the neurons responded to laser stimulation but not to acoustic stimuli.

The following parameters were varied while the activity of the nerve fiber was recorded for 10s time intervals:

-increasing optical energy
-pulse length
-pulse repetition rate

Deafening experiments

During the last quarter we deafened animals by injecting transtympanically sterile Ringer's Lactate containing neomycin of different concentration. The objective of the experiments was to correlate the number of surviving spiral ganglion cells after neural degeneration with the amplitude of the optically evoked compound action potentials. This quarter, we started measuring acoustic threshold curves and optically evoked action potentials in animals which have survived the application of neomycin into the middle ear by four weeks.

Following the experiments the cochleae were harvested and plastic embedded. During the coming quarter the specimen will be sectioned and the spiral ganglion cell density will be measured.

Compound action potential (CAP) measurements in gerbils

Measuring the peak-to-peak amplitude of the optically evoked compound action potential (CAP) from the cochlea serves to monitor cochlear function. The CAP amplitude decreases when cochlear damage occurs. Compound action potentials were recorded at the round window for different stimulus parameters:

-radiation wavelength (1.95 μm)
-pulse length (down to 1μs)
-increasing optical energy

The results show that pulse durations as short as 1 μs elicit compound action potentials from the cochlea. The energy required to evoke a CAP remains the same for pulse durations between 5 – 30 μs. The radiation wavelength used in the current experiments corresponds to a shorter penetration depth than previous experiments. The optical fiber had to be placed closer to the target to evoke an action potential. The results were obtained from normal hearing animals.

Tone-on- light masking

The experiments have been started before the award has been made and have been continued during the first quarter. They will answer some of the questions of Stage I. The experiments determine the spatial selectivity of stimulation with optical radiation, here in the gerbil.

For the present experiments, we have used a masking technique in the gerbil cochlea to determine the frequency selectivity of optical stimulation. CAP masking experiments were conducted with the probe response evoked by light, rather than the conventional probe tone. The placement of the optical fiber determined the probe frequency. A continuous masker tone was presented simultaneously to the light. In a similar manner to tone-on-tone masking, the masker level was determined such that it reduced the laser evoked CAP by 6 dB. From this method, we constructed tuning curves, which we term tone-on-light tuning curves, as a measure of the selectivity of laser stimulation in the cochlea. For comparison, tuning curves were also obtained for tone-on-tone masking and tone-on-electrical masking.

The experiments have been completed and a manuscript to present the results is in preparation.

Guinea pigs

We have proposed that the experimental results obtained in gerbils will be verified, in guinea pigs. Guinea pigs have been purchased and the surgical access has been developed. During the last quarter we were not able to record neural activities. The latter is planned for the next quarter.

Symbiotic activities

Facial nerve measurements

Experiments have been reported during the last quarter exploring the possibility of using the laser to stimulate and identify the facial nerve. The experimental results have been prepared for publication in the Laryngoscope. The paper has been accepted for publication and a copy of the manuscript is attached to this report.

A comment of one of the reviewer's has raised an extremely important issue: Will different tissues affect the transmission and the focus of the beam? Interaction of the laser radiation with the tissue determines the spatial distribution of light in the tissue. Primary light-tissue interactions fall into two broad categories, absorption and scattering, the prevalence of which is highly dependent on the wavelength of the light and the tissue characteristics. [6,7] To a first approximation, absorption dominates the light-tissue interaction in the mid-infrared wavelength range. The axial distribution of a primarily absorbed wavelength can be determined using Beer's Law, a description of the exponential decrease of laser energy over the optical path. However, scattering does play some small role in the light-tissue interaction at mid-infrared wavelengths. Spatial scatter is caused by random spatial variations in tissue density, refractive index, and dielectric constant. While it has been reported that the scattering decreases monotonically with wavelength, a rigorous description of the multiple scattering events that occur as a collimated beam propagate through tissue is extremely difficult.[7]

At present, we have determined that the infrared wavelength of the radiation, which we used to stimulate the facial nerve spreads little in air and in saline (Fig. 1). Although tissue in the radiation path increases the scatter (Fig. 1), the width of the beam is less than a 1 mm at the level of the nerve. The addition of focusing optics could potentially decrease this value to the desired spot size. A more thorough study of this issue is in progress. The full-width of the beam 6 dB below the maximum and the radiation energy will be measured for:

1.) skin
2.) muscle tissue
3.) fatty tissue
4.) bone in general
5.) pig temporal bones
6.) pig bones from the modiolus
7.) human temporal bones
8.) bone samples from the modiolus of human cochleae.

Plan for the next quarter

1. Continue analyzing the data and prepare the single fiber data for publication
2. Analyze and summarize the data obtained in acutely and chronically deafened animals
3. Confirm results from the gerbils in guinea pigs
4. Test the multi-channel system by recording from the guinea pig inferior colliculus.
5. Complete the study on the effect of tissue on the beam profile. We will determine the bulk transmission properties of different tissue and of bone.
6. Prepare the presentation for CIAP.

References

1. Wells JD, Kao C, Mariappan K et al. Optical stimulation of neural tissue in vivo. *Optics Letters* 2005;30:504-506.
2. Richter C-P, Izzo AD, Walsh Jr. JT, Jansen DE. Optical Stimulation of the Auditory System. *Neural Interface Workshop, NIH, Bethesda* 2005.
3. Richter C-P, Izzo A, Walsh J, Jansen DE. Spatial cochlear tuning obtained with optical stimuli. *Conference on Implantable Auditory Prostheses Asilomar* 2005.
4. Izzo A, Richter C-P, Walsh J, Jansen D. Safe ranges for optical cochlear neurons stimulation. *Abstr Assoc Res Otolaryngol* 2005;28:1013.
5. Richter C-P, Izzo A, Walsh J, Jansen D. Optically-evoked Acoustic Nerve Activity. *Abstr Assoc Res Otolaryngol* 2005;28:1012.
6. Niemz MH. *Laser Tissue interactions: fundamentals and application.* New York: Springer, 2004.
7. Welch AJ, van Gemert MJC. *Optical-Thermal Response of Laser-Irradiated Tissue.* New York: Plenum Press, 1995.

Appendix

Figures

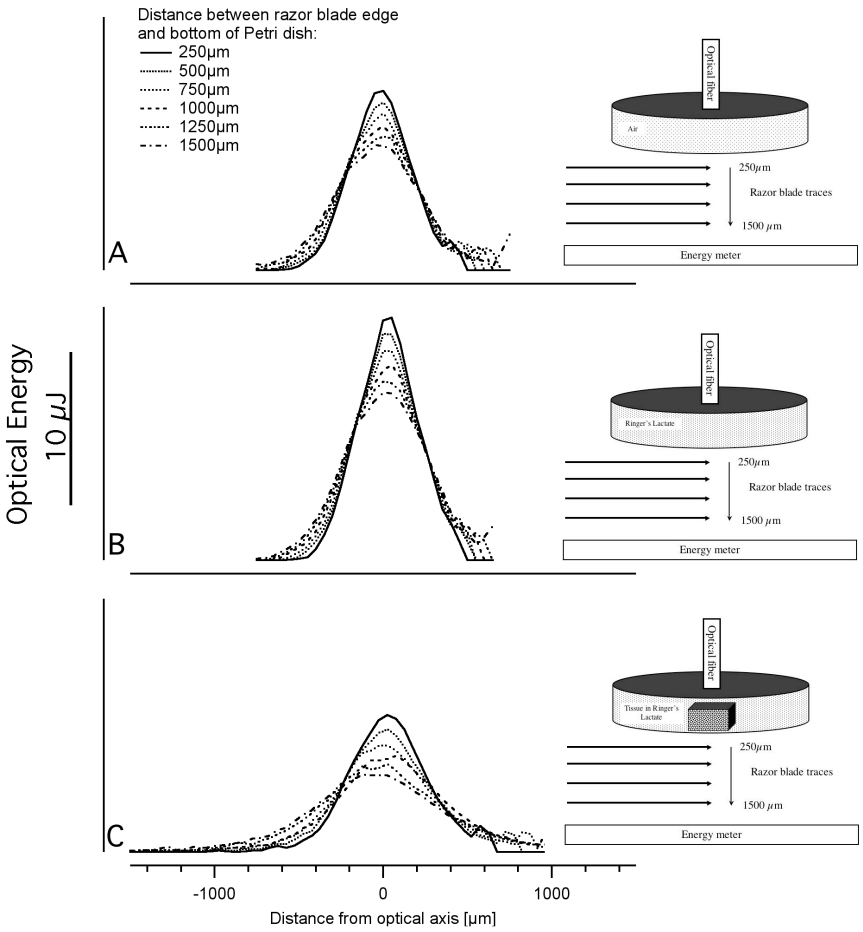

Figure 1: Shown are the measurements of the laser beam profile. The optical fiber, which is coupled to the laser, is placed approximately 2 mm away from the bottom of a Petri dish (left column). The Petri dish contains a thin glass window below the tip of the optical fiber. The radiation energy is measured with an energy sensor below the dish. During the measurements, a razor blade is moved in 50 μm increments into the optical path thereby blocking the radiation to the energy sensor. The razor blade is moved along traces with increasing distance form the dish. The resulting plots show the incremental energy change for each advancement of the razor-blade. The results reveal a gaussian laser beam profile. Measurements were made while the dish was filled with air (A), with Ringer's Lactate (B), and with a 1.5 mm thick slice of muscle tissue immersed in Ringer's Lactate placed below the optical fiber (C). The laser spot size becomes wider when tissue is placed between the optical fiber and the energy sensor.

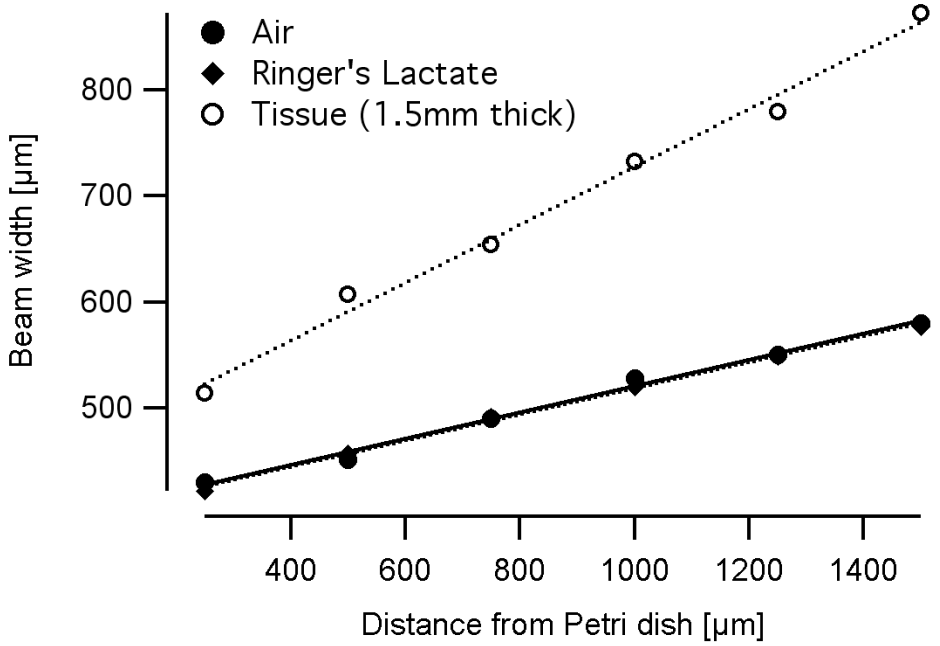

Figure 2: Traces show the width of the laser beam for different conditions described in Figure 6. The width of the beam was determined as the full width at 6 dB below the maximum optical energy for each of the traces. The width is very similar for the beam in air and in Ringer's Lactate. Tissue in the optical path, however, widens the beam.